Patterns of nucleotide polymorphism distinguish temperate and tropical wild isolates of Caenorhabditis briggsae

DOI: 10.1534/genetics.106.058651

Patterns of Nucleotide Polymorphism Distinguish Temperate and Tropical Wild Isolates of *Caenorhabditis briggsae*

Asher D. Cutter,*[,1] Marie-Anne Félix,[†] Antoine Barrière[†] and Deborah Charlesworth*

**Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom and [†]Institut Jacques Monod, Centre National de la Recherche Scientifique, Universities Paris 6/7, Paris, France*

Manuscript received March 30, 2006
Accepted for publication June 5, 2006

ABSTRACT

Caenorhabditis briggsae provides a natural comparison species for the model nematode *C. elegans*, given their similar morphology, life history, and hermaphroditic mode of reproduction. Despite *C. briggsae* boasting a published genome sequence and establishing Caenorhabditis as a model genus for genetics and development, little is known about genetic variation across the geographic range of this species. In this study, we greatly expand the collection of natural isolates and characterize patterns of nucleotide variation for six loci in 63 strains from three continents. The pattern of polymorphisms reveals differentiation between *C. briggsae* strains found in temperate localities in the northern hemisphere from those sampled near the Tropic of Cancer, with diversity within the tropical region comparable to what is found for *C. elegans* in Europe. As in *C. elegans*, linkage disequilibrium is pervasive, although recombination is evident among some variant sites, indicating that outcrossing has occurred at a low rate in the history of the sample. In contrast to *C. elegans*, temperate regions harbor extremely little variation, perhaps reflecting colonization and recent expansion of *C. briggsae* into northern latitudes. We discuss these findings in relation to their implications for selection, demographic history, and the persistence of self-fertilization.

QUANTIFICATION of population genetic variation in nature is key to understanding the forces responsible for shaping evolutionary change. The processes of mutation, drift, recombination, demography, and selection each likely contribute to patterns of genetic variation in Caenorhabditis nematodes, although their relative strength within and among species remains unclear. The nematode *Caenorhabditis elegans*, a model for diverse areas of biology, has a recent but growing literature in population genetics (Graustein *et al.* 2002; Jovelin *et al.* 2003; Sivasundar and Hey 2003; Barrière and Félix 2005; Haber *et al.* 2005; Sivasundar and Hey 2005; Cutter 2006). The evolutionary context of population processes in *C. elegans* will be greatly informed by understanding patterns of genetic variation in related species, for which relatively few data are available (Thomas and Wilson 1991; Graustein *et al.* 2002; Jovelin *et al.* 2003). This notion of the benefits of comparative work motivated the recent genome sequencing of the congener *C. briggsae* and ongoing sequencing projects for other species of Caenorhabditis (Stein *et al.* 2003). Indeed, genetic mapping and mutant generation efforts in *C. briggsae* are developing Caenorhabditis into a model genus for which *C. elegans* is a convenient stepping stone. Morphologically, these two species are exceedingly similar, with only subtle differences in traditional systematic characters, such as morphology of the male tail and excretory pore (Nigon and Dougherty 1949; Fitch and Emmons 1995; Baird 2001; Wang and Chamberlin 2002; Félix 2004), despite an estimated divergence of many millions of years (Coghlan and Wolfe 2002; Stein *et al.* 2003). *C. briggsae* shares the same selfing mode of reproduction as *C. elegans* and is androdioecious, although phylogenetic analyses and the molecular genetics of sex determination argue that the similar mating systems probably evolved independently (Kiontke *et al.* 2004; Nayak *et al.* 2005; Hill *et al.* 2006). These two species also have partially sympatric geographic distributions and were isolated together on several occasions, although species ranges are poorly characterized in both species. More importantly for molecular evolutionary work, other currently known species in the genus share common ancestors more recently with *C. briggsae* than with *C. elegans* (Kiontke *et al.* 2004; Kiontke and Sudhaus 2006). In addition, recent work in *C. briggsae* has shown heritable variation for male reproductive structures (Baird *et al.* 2005) and cell lineage properties of hermaphrodite vulva development (Delattre and Felix 2001). Some cross-species hybrids form partially developed embryos (Baird *et al.* 1992; Baird and Yen 2000; Hill and L'Hernault 2001; Baird 2002), potentially permitting a Caenorhabditis model for speciation. These factors make it desirable to acquire a general

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. DQ632004–DQ632387.

[1]*Corresponding author:* Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON M5S 3G5, Canada.
E-mail: acutter@eeb.utoronto.ca

TABLE 1

***C. briggsae* strains included in this study**

Strains	Location (nearest city)	Latitude (N)	Source
VT847	Hawaii, United States	20° 57′	CGC
JU726	Chengyang, Guandong, China	21° 13′	This study
AF16	Ahmedabad, India	23° 01′	CGC
JU725	Yangshuo, Guangxi, China	24° 46′	This study
ED3032	Taipei, Taiwan, China	25° 02′	This study
ED3033–ED3037	Taipei, Taiwan, China	25° 02′	This study
HK104	Okayama, Hirusen, Japan	34° 40′	CGC
HK105	Sendai, Aobayama, Japan	38° 16′	CGC
PB826	Hueston Woods St. Pk., OH, United States	39° 34′	CGC
PB800	Dayton, OH, United States	39° 53′	CGC
JU516	Marsas, France	43° 02′	This study
JU793–JU797	Frechendets, France	43° 04′	This study
JU348–JU358	Merlet, France	44° 26′	This study
JU757	Le Blanc, France	46° 37′	This study
JU441	Beauchene, France	47° 55′	This study
JU835–JU846	Obernai, France	48° 27′	This study
JU279, JU280, JU296	Paris, France	48° 50′	This study
JU372–JU377, JU379–JU383	Viosne Valley, Oise, France	49° 04′	This study
JU403–JU405	Hermanville, France	49° 17′	This study
JU439	Reykjavik, Iceland	64° 08′	This study

CGC, Caenorhabditis Genetics Center.

understanding of the patterning of natural genetic variation in *C. briggsae*.

Understanding population genetic variation helps to illuminate the scope of selective, neutral, and demographic processes shaping genomes. One important factor in Caenorhabditis, as well as in many other groups of animals and plants (Jarne and Charlesworth 1993), is the presence of self-fertilization. Self-fertilization can profoundly affect how new mutations (beneficial, detrimental, and neutral) spread in a species through its effects on increased homozygosity, reduced genetic variation, reduced effective recombination, and population subdivision (Charlesworth 2003). Population structure induced by selfing can also affect the fixation probabilities for newly arising alleles through dependencies on interdemic migration rates and colonization–extinction dynamics, which influence effective population sizes (Wade and McCauley 1988; Pannell and Charlesworth 1999; Wakeley and Aliacar 2001; Pannell 2003; Peters *et al.* 2003). Ecologically, self-fertilizing hermaphrodites are able to colonize new habitats as solitary individuals but may also be subject to inbreeding depression (Baker 1955; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). Thus, self-fertilization can result in a cascade of genetic consequences with important implications for adaptation and genome evolution.

In an effort to characterize natural variation in the self-fertile *C. briggsae*, we have collected 57 new isolates from two continents, for which we assayed nucleotide polymorphism at six loci. Surveys of diversity that use nucleotide sequence data provide several advantages over other common measures of genetic variation by using well-defined regions that are expected to conform to known mutational dynamics. Consequently, the extensive population genetic theory that has developed for such variants can be applied to the resulting data and, given the developing resources in *C. briggsae*, analysis of known regions allows integration with genomic information and a window into genome evolution. The pattern of polymorphisms in this study reveals differentiation between *C. briggsae* strains found in temperate localities in the northern hemisphere and those sampled near the Tropic of Cancer, with diversity within the tropical region comparable to what is found for *C. elegans* in Europe. In contrast with *C. elegans*, exceptionally little polymorphism is present among European samples of *C. briggsae*, possibly reflecting colonization and recent expansion of *C. briggsae* in temperate latitudes. However, broader and denser geographic sampling of both *C. briggsae* and *C. elegans* is necessary to clearly define the full extent of their sympatry, population subdivision, and genetic diversity.

MATERIALS AND METHODS

Nematode populations: We include 63 isohermaphrodite strains of *C. briggsae* in this study, including 6 obtained from the Caenorhabditis Genetics Center (Table 1; Figure 1). The majority of these strains were collected from eight sites across France (Frechendets, Paris, Hermanville, Merlet, Marsas, Beauchene, Obernai, Viosne Valley) from compost heaps (Marsas, Frechendets, Le Blanc, Hermanville), snails (under mulberry tree, *Oxychilus* sp., Merlet; rotting *Helix aspersa*,

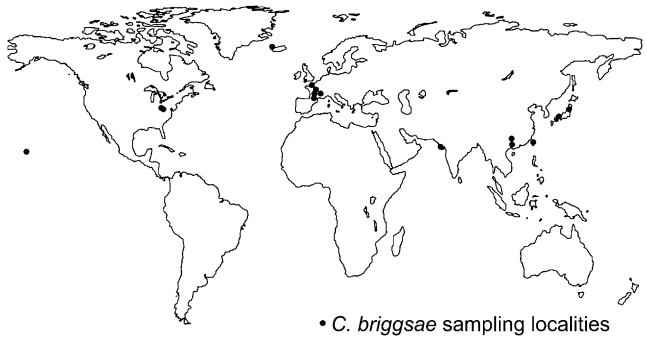

Figure 1.—Geographic distribution of *C. briggsae* sampling sites.

Paris), soil and detritus from a vegetable garden (Obernai), soil below a mulberry tree (Merlet), and soil from a road embankment along a cultivated field (Viosne Valley). Nematodes were isolated and identified as previously described (Barrière and Félix 2005), with developmental timing consistent with their having been in the dauer stage at the time of collection in most cases, with the exception of the sample from Obernai in which all stages were present. We also isolated one new strain in Reykjavik, Iceland (a cemetery flower bed), two new strains from mainland China (mushroom compost in farmyard, JU725; soil and compost in cabbage field, JU726), and six new strains in Taipei, Taiwan (China) (Table 1). The new Taiwanese strains derive from two localities within Taipei: a piece of rotting wood from a private garden in Tienmu (ED3033–ED3037) and a sample from a flower bed in a public garden in Chungcheng (ED3032). Individual nematodes were isolated from the samples, with species identity confirmed with mating tests or sequencing of the small-subunit ribosomal RNA gene (Floyd *et al.* 2002). Strains are available upon request (http://www2.ijm.jussieu.fr/worms).

Molecular methods and genetics: DNA was isolated using an NaOH digestion protocol for single individuals or groups of ≤5 individuals for each of the 63 strains of *C. briggsae* (Floyd *et al.* 2002). We selected for resequencing in these strains the putative orthologs of six genes from *C. elegans* chromosomes II and X that were assessed for polymorphism previously in *C. elegans* (Cutter 2006). Primers were designed to span a long (>500 bp) intron in each of these loci on the basis of Wormbase annotations for *C. briggsae* (Table 2). However, because of evolutionary changes in intron size and location, the specific *C. briggsae* regions that were sequenced are not all homologous with the *C. elegans* introns sequenced by Cutter (2006), despite lying within orthologous genes. In the case of one *C. elegans* gene (D1005.1), a convincing ortholog in *C. briggsae* with adequate primers could not be found, so the putative ortholog of a nearby *C. elegans* locus was used instead (*C. briggsae* CBG24509 homologous to *C. elegans* F09E10.5). Both strands were sequenced on an ABI 3730 automated sequencer by the University of Edinburgh sequencing facility.

Because a fully integrated genetic map with contiguous genome sequence is not yet available for *C. briggsae*, we mapped these loci relative to each other. Restriction and length polymorphisms in four of the five polymorphic loci allowed us to score genotypes in a set of 94 recombinant inbred lines (RIL) and in their parental strains (AF16 and HK104) that are being used for the *C. briggsae* mapping project (http://snp.wustl.edu/snp-research/c-briggsae; supplemental Table 1 at http://www.genetics.org/supplemental/); the RIL were constructed by S. Baird, who kindly provided DNA samples. We then inferred linkage groups and recombination distances between

TABLE 2

Feature statistics of sequenced loci

Locus ID	*C. briggsae* gene	Ultra-contig	Super-contig	*C. elegans* ortholog	Forward primer	Reverse primer	Size
p09	CBG19635	cb25.fpc4131	c001701154.Contig2	Y25C1A.5 (II)	TTGATAAGCCAACCCCAGTC	GAGATTGGCAGCAAGGAATC	843
p10	CBG03684	cb25.fpc0071	c009701346.Contig1	ZK430.1 (II)	TGGAAATGTGGATGCGAGTA	TTGAAGTCGTCCCAGGATTC	779
p11	CBG20775	cb25.fpc4206	c002000737.Contig8	E01G4.6 (II)	AATCCAGCAAGGTCTCTCCA	TCCACGTTGGATGACAAGAA	863
p12	CBG14460	cb25.fpc3857	c000600869.Contig1	R160.7 (X)	TTGGAATAAACCTCGTCCAGA	GATCCTGAGCCACACGAAGT	787
p13	CBG16368	cb25.fpc4033	c005600403.Contig1	T24D11.1 (X)	AAAAAGCGTCCGAATCACAT	GGAATATCGCGTTGGATGAC	792
p14	CBG24509	cb25.NA_175	c013101146.Contig1	F09E10.5 (near D1005.1) (X)	TCATGACCAACAAAGAAATGC	TCAACCAAGAACGTCTCGAA	777

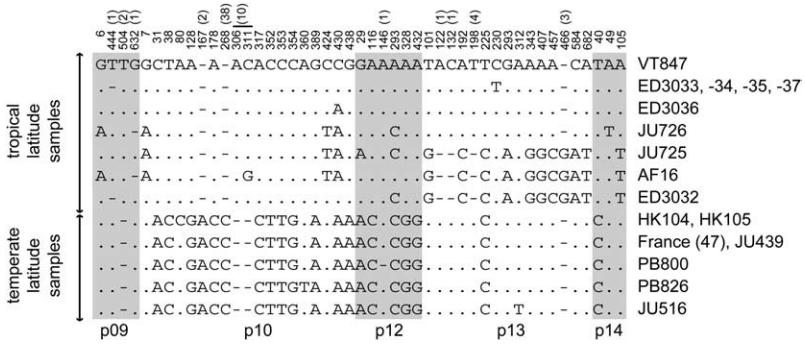

FIGURE 2.—Summary of sequenced regions, polymorphisms, and haplotypes. Loci are indicated along the bottom, demarcated by alternating backgrounds (p12 and p13 localize to the X chromosome, p10 to chromosome II). Positions of polymorphisms within the sequence for each locus are listed across the top with indel lengths in parentheses. Only the first base is shown for indel polymorphisms, except for p10, in which an SNP overlaps an indel. Strain names for each haplotype are listed along the right; strains for 47 identical French haplotypes are given in Table 1.

loci using the Kosambi mapping function in MapManager QTX b20 (http://www.mapmanager.org).

Sequence analysis: Sequencher v.4.0 and BioEdit were used for sequence alignment and manual editing to confirm sequence quality and to remove primer sequences. Calculations of diversity from pairwise differences (π) and from the number of segregating sites (θ) (WATTERSON 1975; NEI and LI 1979), linkage disequilibrium, recombination, and tests of neutrality were performed using DnaSP v.4.10.04 (ROZAS *et al.* 2003), RecMin (MYERS and GRIFFITHS 2003), and LIAN v.3.1 (HAUBOLD and HUDSON 2000). We focus on diversity at silent sites (synonymous and intronic positions; π_{si} and θ_{si}), which compose the majority of the data set. Sites associated with indels or incomplete data were excluded from the analyses. Neighbor-joining trees were constructed with PAUP* v.4.0 using concatenated sequences, but should not be used to infer phylogenetic relationships between strains because of the evidence for recombination in these data (see below). Consequently, we also used SplitsTree4 (HUSON and BRYANT 2006) to graphically represent the relationships between haplotypes so as to include evidence for recombination.

RESULTS

In 4164 bp of *C. briggsae* sequence across six loci, we identified 35 single nucleotide polymorphisms, 11 indel polymorphisms, plus an SNP that occurred within an indel (Figure 2). Overall silent-site nucleotide polymorphism is low, with π_{si} and θ_{si} estimated to be ~0.2% among tropical samples (Table 3). No polymorphisms were found in the 789 bp of coding sites (182.67 of which are synonymous) or in the 738 bp surveyed for locus p11. The variants include 18 transitions and 17 transversions, corresponding to a low transition:transversion ratio (ts/tv) of 1.06, a nonsignificant difference from the lower bound estimate for ts/tv in *C. elegans* (1.3–1.6, binomial $P = 0.068$) (KOCH *et al.* 2000; DENVER *et al.* 2003, 2004; CUTTER 2006). Length variants were present in four of the six loci and include five single-base indels, two double-base indels, and individual indels 3 and 4 bp in length (Figure 2). An additional indel complex in locus p10 appears to be composed of 10- and 38-bp indels as well as a single nucleotide polymorphism. Some of these indels were associated with simple repeats, including two indels forming part of an $A_{3-4}C_{5-6}$ motif in p10, an A_{7-8} motif in p12, and two indels as part of an $A_{7-8}C_{2-3}$ motif in locus p13.

An exceptionally low level of polymorphism is represented among the 53 samples from temperate latitudes (34–64° N: France; Iceland; Japan; Ohio, United States), including only three polymorphic sites with a corresponding pairwise silent-site diversity estimate of $\pi_{si} = 0.00004$. Indeed, 48 isolates from eight sites across France and Iceland all share the same multilocus haplotype (Figure 2). This finding was a surprise, given

TABLE 3

Summary of *C. briggsae* nucleotide polymorphism

	Diversity: π_{si}			Diversity: θ_{si}			Segregating sites: S		
Locus ID	Tropical[a]	Temperate[b]	CGC Strains[c]	Tropical[a]	Temperate[b]	CGC Strains[c]	Tropical[a]	Temperate[b]	CGC Strains[c]
p09	0.00072	0	0.00067	0.00071	0	0.00088	1	0	1
p10	0.00285	0.00018	0.01251	0.00241	0.00072	0.01141	4	2	15
p11	0	0	0	0	0	0	0	0	0
p12	0.00106	0	0.00425	0.00112	0	0.00349	2	0	5
p13	0.00790	0.00006	0.00498	0.00590	0.00036	0.00655	10	1	9
p14	0.00111	0	0.00144	0.00118	0	0.00146	2	0	2
Concatenated	0.00232	0.00004	0.00404	0.00193	0.00019	0.00403	19	3	32
Average	0.00227	0.00004	0.00398	0.00189	0.00018	0.00397	3.2	0.5	5.3

[a] Ten samples from latitudes ≤25°N.
[b] Fifty-three samples from latitudes ≥34°N.
[c] Six samples from the Caenorhabditis Genetics Center used in GRAUSTEIN *et al.* (2002).

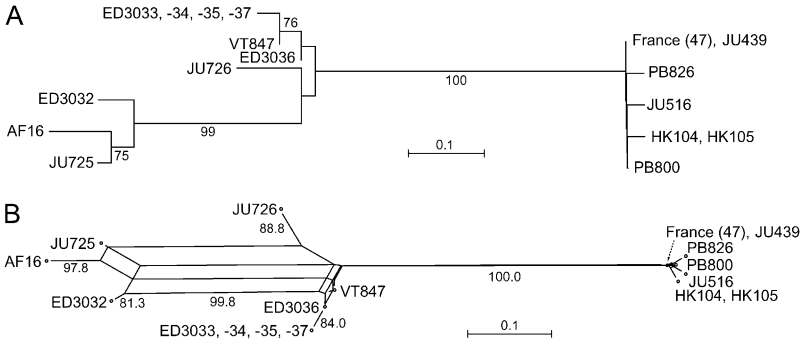

FIGURE 3.—Unrooted *p*-distance neighbor-joining tree (A) and neighbor network (B) of *C. briggsae* multilocus haplotypes. Reticulation in B indicates possible recombination between haplotypes. Numbers above or below a branch indicate bootstrap support percentages ≥75% out of 1000 replicates. Strain designations are as in Table 1.

the normal levels of diversity observed for *C. elegans* in similar samples in some of the same locations (BARRIÈRE and FÉLIX 2005; CUTTER 2006). In contrast, the 10 more tropical samples (20–25° N: China; India; Hawaii, USA) harbor nearly all of the diversity identified in the species ($\pi_{si} = 0.0023$). Given our structured sampling scheme among the French collections, it would seem appropriate to use a subsampling method to estimate diversity (LESSARD and WAKELEY 2004); however, the near absence of polymorphism in these samples limits the utility of such an approach. Nevertheless, inclusion of only a single individual from each temperate locality in calculating diversity yields $\pi_{si} = 0.00016$ ($\theta_{si} = 0.00027$). In addition to the difference in overall levels of diversity between temperate and tropical isolates, haplotype patterns clearly distinguish *C. briggsae* between tropical and temperate regions. There are 14 fixed single nucleotide differences and three indels from four different loci differentiating the temperate from tropical strains. This temperate–tropical dichotomy is reflected clearly both in the table of polymorphisms and in a neighbor-joining tree of haplotypes (Figures 2 and 3). Although genealogies with two long ancestral branches are typical of neutral genealogies, particularly for highly inbreeding species, such genealogies are generally not expected to be associated with geography or to partition the same individuals repeatedly for different loci.

The clear differentiation between isolates from temperate and tropical localities led us to explore other features of the polymorphism data separately for these two classes of samples. In particular, we were interested in whether or not the samples showed evidence for departure from a neutral equilibrium scenario with no selection, population structure, or changes in population size. By comparing diversity estimates on the basis of pairwise differences (π) or the number of segregating sites (θ), we quantified departures from the neutral expectation with Tajima's *D* and Fu and Li's *D** statistics (WATTERSON 1975; NEI and LI 1979; TAJIMA 1989; FU and LI 1993). The two diversity estimators (π and θ) should yield similar values at neutral equilibrium, resulting in values of *D* and *D** that approach zero, whereas non-neutral demographic processes affect θ and π differently and can lead to positive (*e.g.*, population contraction or structure) or negative (*e.g.*, population growth) *D* and *D**. For the 10 tropical samples, nominal values of *D* (and *D**) were positive on average (mean $D = 0.37$, $D^* = 0.43$; Table 4), with locus p13 significantly so ($D^* = 1.46$, $P < 0.05$). The low overall diversity makes it difficult to ascertain whether there is a general trend of positive skew in the frequency spectrum (*i.e.*, $D > 0$, a deficit of rare alleles); if so, then this suggests that population structure or a population contraction may underlie part of the pattern of variation in the tropical samples (CHARLESWORTH *et al.* 1993), although our sampling of low-latitude regions is too sparse to test these ideas more rigorously. In contrast to the tropical samples, the two polymorphic loci among the temperate samples both exhibit nonsignificant negative values for *D* and *D** (Table 4). Coupled with the extreme lack of polymorphism among the European, Japanese, and continental United States samples, this might correspond to a recent colonization of northerly latitudes followed by population expansion.

The pattern of polymorphisms for these six loci indicates that recombination has occurred in the history of the sample. Using a conservative method based on the four-gamete test (HUDSON and KAPLAN 1985), a minimum of $R_m = 2$ recombination events is evident, whereas an alternative approach suggests a minimum of

TABLE 4

Skews in the frequency spectrum for tropical and temperate samples

	Tajima's *D*		Fu and Li's *D**	
Locus ID	Tropical	Temperate	Tropical	Temperate
p09	0.01		0.80	
p10	0.69	−1.31	0.45	−0.91
p11				
p12	−0.18		−0.28	
p13	1.50	−1.09	1.46[a]	−1.86
p14	−0.18		−0.28	
Concatenated	0.96	−1.58	0.93	−1.75
Average	0.37	−1.20	0.43	−1.39

[a] $P < 0.05$.

TABLE 5

Average linkage disequilibrium (r^2) between each pair of loci

	Mean r^2				
Locus ID	p09	p10	p12	p13	p14
p09	NA				
p10	0.497	0.495			
p12	0.038	0.178	0.259		
p13	0.059	0.238	0.258	0.857	
p14	0.246	0.204	0.201	0.489	0.048

NA, not applicable.

$R_h = 3$ recombination events (Myers and Griffiths 2003). In addition to the historical recombination predicted to have occurred between loci (p10–p12, p12–p13), the Myers and Griffiths (2003) method predicts intralocus recombination for locus p12. We also quantified linkage disequilibrium between pairs of polymorphic sites among the samples collected in tropical regions. Although the average values of the r^2 measure of linkage disequilibrium were high both within and between loci (Table 5), no individual pair of sites deviated significantly for Fisher's exact tests after applying the conservative Bonferroni correction for multiple tests, given only 10 samples. However, a multilocus statistic that considers all sites together ($I_A = 0.232$; Haubold and Hudson 2000) identifies significant linkage disequilibrium overall ($P = 0.01$), despite the lack of power to detect specific pairs of sites with significant linkage disequilibrium.

Our linkage analysis, in which we scored restriction polymorphisms in a set of recombinant inbred lines, allowed us to place loci p12 and p13 on the same linkage group, separated by ~27.1 cM. From the locations of ultra-contigs for p12 and p13 on the preliminary *C. briggsae* SNP map v.3.1 (http://snp.wustl.edu/snp-research/c-briggsae/), we infer that they reside on the X chromosome like their orthologs in *C. elegans*. Two other loci (p09, p10) showed independent segregation, and we were unable to score the remaining two loci (no polymorphic sites in p11, no polymorphic restriction sites in p14). However, super-contig locations for both p10 and p11 on the SNP map indicate locations on *C. briggsae* chromosome II, with a separation of ~22.3 cM (the *C. elegans* orthologs lie on chromosome II).

Given that mutation accumulation experiments suggest that *C. elegans* and *C. briggsae* have roughly similar mutation rates, although the rate in *C. briggsae* may be somewhat higher (Baer *et al.* 2005), we can apply direct estimates of the *C. elegans* neutral mutation rate to the *C. briggsae* diversity information to infer its effective population size (N_e). Assuming that *C. briggsae* populations are at equilibrium (*i.e.*, $\theta = 4N_e\mu$) and that the neutral mutation rate $\mu = 9.0 \times 10^{-9}$ (Denver *et al.* 2004), we estimate the effective population size of *C. briggsae* in tropical regions to be $\sim 6 \times 10^4$. If the neutral mutation rate in *C. briggsae* is higher than that of *C. elegans* and if population structure is present in the tropical samples, then this value of N_e will be an overestimate (Baer *et al.* 2005).

DISCUSSION

Patterns of nucleotide polymorphism: The global pattern of polymorphism in *C. briggsae* reveals a small set of highly similar haplotypes in temperate regions that are distinct from the bulk of the known species diversity present in tropical latitudes. Samples from localities around the Tropic of Cancer are characterized by low levels of silent-site nucleotide diversity, averaging $\pi_{si} = 0.2\%$, whereas temperate samples exhibit exceedingly little diversity with $\pi_{si} = 0.004\%$. Moreover, fixed differences in four loci clearly differentiate these two classes of samples.

A previous study of polymorphism for two nuclear genes in *C. briggsae* reported much higher overall levels of diversity than we find for tropical samples (*tra-2* $\pi_{si} = 0.53\%$, *glp-1* $\pi_{si} = 0.42\%$; Graustein *et al.* 2002), whereas data from another study in a sample of four strains yielded a diversity estimate similar to those reported here (*odr-3* $\pi_{si} = 0.19\%$; Jovelin *et al.* 2003). We recalculated π and θ using the subset of six strains in our sample that were used by Graustein *et al.* (2002) and recapitulate their pattern of higher diversity estimates (Table 3). However, these higher values probably reflect a classic effect of population subdivision due to the presence of differentiated tropical and temperate strains in the same sample (Charlesworth 2003). Nevertheless, this differentiation indicates that global species diversity is greater than the 0.2% observed within available tropical samples.

Linkage disequilibrium and recombination: Overall multilocus linkage disequilibrium is greater than that expected by chance, with high magnitudes of pairwise linkage disequilibrium within and between loci. Despite the presence of linkage disequilibrium within loci, the pattern of polymorphisms also reveals a history of recombination. Depending on the method used, these data indicate the presence of at least two to three recombination events in the history of the sample (Hudson and Kaplan 1985; Myers and Griffiths 2003). Using an approach previously described for *C. elegans* data (Cutter 2006), we can attempt to roughly infer levels of outcrossing and selfing from interlocus linkage disequilibrium. For the four polymorphic loci that have mapping information (with 50 cM separating independently segregating loci), the levels of linkage disequilibrium among tropical strains imply an outcrossing rate of $\sim 3.9 \times 10^{-5}$ (range of values from average LD between locus pairs: 4.2×10^{-6}–1.1×10^{-4}), assuming that all of the observed linkage disequilibrium is due to self-fertilization and that $N_e = 6 \times 10^4$. This amount of outcrossing is similar to, but slightly lower

than, the low levels inferred from linkage disequilibrium in *C. elegans* (Barrière and Félix 2005; Cutter 2006), and alternative methods have resulted in even higher outcrossing rate estimates in *C. elegans* (Barrière and Félix 2005; Sivasundar and Hey 2005). If population structure is present within the tropical sample, then this outcrossing rate estimate may be downwardly biased, although it is possible to remove an effect of structure by sampling single individuals from different subpopulations (Lessard and Wakeley 2004; Cutter 2006). However, it may be expected that *C. briggsae* will experience lower outcrossing rates than *C. elegans*, because X-bearing male sperm fertilize oocytes more readily than do nullo-X male sperm in *C. briggsae* (Lamunyon and Ward 1997), which is likely to decrease equilibrium male frequency and outcrossing (Chasnov and Chow 2002; Stewart and Phillips 2002; Cutter *et al.* 2003a).

Comparison with *C. elegans*: Unlike the distribution of genetic diversity observed in *C. elegans*, a clear geographic partitioning of variation is evident for *C. briggsae* (Barrière and Félix 2005; Haber *et al.* 2005; Cutter 2006). This patterning also is evident in the survey of Graustein *et al.* (2002), although their sample of only six *C. briggsae* strains precluded any biogeographic inference about the few haplotypes observed. The differentiation among temperate and tropical latitudes in *C. briggsae* leads to greater maximum divergence between intraspecific haplotypes in *C. briggsae* than in *C. elegans* ($K_{si} = \sim 0.84\%$ *vs.* $\sim 0.37\%$), although variation within the tropical samples is comparable to polymorphism among European *C. elegans* ($\pi_{si} = \sim 0.2\%$; Cutter 2006). Variation at orthologous loci is not correlated in the two species. By contrast to European *C. elegans*, genetic variation is virtually absent among *C. briggsae* samples within Europe. For the two French localities in which more than one isolate each of *C. elegans* and *C. briggsae* were collected, no polymorphism is present for *C. briggsae*, whereas for *C. elegans* diversity levels are typical (Merlet $\pi_{si} = 0.12\%$ and Hermanville $\pi_{si} = 0.26\%$; Cutter 2006). This lack of diversity among temperate *C. briggsae* isolates, coupled with the few variants being present primarily as singletons, suggests that this species may have colonized and expanded in temperate latitudes more recently or from fewer sources than *C. elegans*. Unfortunately, the present density of sampling of *C. briggsae* within tropical regions is insufficient to test for population subdivision, which has been observed for *C. elegans* among sampling localities (Barrière and Félix 2005; Haber *et al.* 2005; Sivasundar and Hey 2005; Cutter 2006).

In more general terms, nucleotide polymorphism is low and quantitatively similar in *C. briggsae* and *C. elegans*. By comparison, the outcrossing *C. remanei* appears to harbor many times higher levels of genetic variation (Graustein *et al.* 2002; Jovelin *et al.* 2003; A. D. Cutter, unpublished data), as do other nematode and Drosophila species (Table 6) (Anderson *et al.* 1998; Andolfatto 2001). Graustein *et al.* (2002) argued that the differences in diversity among Caenorhabditis species is too great to be explained solely by the effects of alternative breeding systems and, therefore, that repeated bottlenecks and/or the action of widespread background selection or genetic hitchhiking must be responsible for the discrepancy. The distribution of single nucleotide polymorphisms with respect to recombination rate in *C. elegans* is suggestive of the action of background selection in the genome (Cutter and Payseur 2003b). Another recent hypothesis regarding demographic effects on *C. elegans* genetic variation holds that extinction–recolonization dynamics could generate extremely reduced levels of diversity (Sivasundar and Hey 2005), although it is not clear whether this is likely to be an important process globally given the extent of migration (Barrière and Félix 2005; Cutter 2006). An additional scenario that might explain exceptionally low variation in *C. elegans* and *C. briggsae* is a recent origin of self-fertilization from a single or small set of progenitors, in which case insufficient time may have elapsed for equilibrium levels of polymorphism to have accumulated. Unfortunately, currently available evidence is insufficient to rule out any of these non-mutually exclusive possibilities.

Implications for *C. briggsae* evolution: What could have led to the differentiation between samples from temperate and tropical latitudes? One possibility is that *C. briggsae* colonized and recently expanded within temperate latitudes from a small founding population. Consistent with this scenario, the Tajima's *D* measure of deviation from neutral expectations tends to be negative in temperate samples, which would suggest population expansion if it is a general pattern in the genome (Tajima 1989). Should this scenario of a relatively recent founding and expansion of *C. briggsae* in temperate regions accurately describe its history, then it is informative to calculate how long ago it may have occurred. For a 60-day generation time (presuming that Caenorhabditis spend most of their lives as dauer larvae, Barrière and Félix 2005) and temperate-region $N_e = 1000$, a coalescent time of $4N_e$ generations (with standard deviation also $\sim 4N_e$ generations; Li 1997) implies that the time to the most recent common ancestor in temperate samples was ~700 years ago with a two standard deviation upper bound of ~2200 years ago (given $\theta = 0.00004 = 4N_e\mu$, where $\mu = 9 \times 10^{-9}$). However, this estimate is subject to an equilibrium assumption that is clearly violated under a scenario of population expansion; the above coalescent time calculation will be an overestimate if the demographic history of the temperate population involves recent growth. Consequently, it seems plausible that *C. briggsae* may have colonized temperate parts of the world only in the past few hundred years, perhaps in association with human activity. However, it is important to point out

TABLE 6

Summary of average nucleotide and microsatellite polymorphism levels for nematode species

Species	Location	π_{si}[a]	H[b]	Reference
Ascaris suum/lumbricoides	Mitochondria	0.016		Anderson *et al.* (1993)
Caenorhabditis briggsae	Mitochondria	0.044		Graustein *et al.* (2002)
Caenorhabditis briggsae	Nucleus	0.002		Graustein *et al.* (2002); Jovelin *et al.* (2003); this study
Caenorhabditis elegans	Mitochondria	0.043		Graustein *et al.* (2002)
Caenorhabditis elegans	Nucleus	0.002	0.62	Graustein *et al.* (2002); Sivasundar and Hey (2003); Barrière and Félix (2005); Haber *et al.* (2005); Cutter (2006)
Caenorhabditis remanei	Mitochondria	0.105		Graustein *et al.* (2002)
Caenorhabditis remanei	Nucleus	0.047		Graustein *et al.* (2002); Jovelin *et al.* (2003); Haag and Ackerman (2005); A. D. Cutter (unpublished data)
Dictyocaulus viviparus	Mitochondria	0.002		Hu *et al.* (2002)
Globodera pallida	Nucleus		0.47	Picard *et al.* (2004)
Haemonchus contortus	Nucleus	0.093		Beech *et al.* (1994)
Haemonchus contortus	Mitochondria	0.026		Blouin *et al.* (1995)
Haemonchus placei	Mitochondria	0.019		Blouin *et al.* (1995)
Heligmosomoides polygyrus	Mitochondria	0.02		Nieberding *et al.* (2005)
Heterodera schachtii	Nucleus		0.58	Plantard and Porte (2004)
Heterorhabditis marelatus	Mitochondria	0.0025		Blouin *et al.* (1999)
Howardula aoronymphium	Mitochondria	0		Jaenike (1996)
Longidorus biformis	Nucleus	0.006		Ye *et al.* (2004)
Longidorus biformis	Nucleus	0.109		Ye *et al.* (2004)
Longidorus diedecturus	Nucleus	0.008		Ye *et al.* (2004)
Longidorus grandis	Nucleus	0		Ye *et al.* (2004)
Longistriata caudabullata	Mitochondria	0.012		Brant and Orti (2003)
Mazamastrongylus odocoilei	Mitochondria	0.028		Blouin *et al.* (1995)
Meloidogyne arenaria/incognita/javanica	Mitochondria	0.0028		Hugall *et al.* (1994)
Necator americanus	Mitochondria	0.012		Hawdon *et al.* (2001)
Onchocerca volvulus	Mitochondria	0.0001		Keddie *et al.* (1999)
Ostertagia ostertagi	Mitochondria	0.025		Blouin *et al.* (1992); Blouin *et al.* (1995)
Strongyloides ratti	Mitochondria	0.001		Anderson *et al.* (1998)
Teladorsagia circumcincta	Mitochondria	0.024		Blouin *et al.* (1995)
Teladorsagia circumcincta	Mitochondria	0.022		Braisher *et al.* (2004)
Teladorsagia circumcincta	Mitochondria	0.022		Leignel and Humbert (2001)
Xiphinema americanum	Nucleus	0.003		Ye *et al.* (2004)
Xiphinema americanum	Nucleus	0.019		Ye *et al.* (2004)
Xiphinema bakeri	Nucleus	0		Ye *et al.* (2004)
Xiphinema index	Nucleus		0.39	He *et al.* (2003)

[a] Nucleotide diversity.
[b] Microsatellite heterozygosity.

that the source of such a founder event is not clear, given the highly distinct temperate-region haplotypes relative to haplotypes known from available tropical samples. Additional sampling of *C. briggsae* in tropical regions will be necessary to elucidate the likely source population that gave rise to the present inhabitants of more temperate latitudes and to determine whether the dichotomous pattern of interstrain relationships persists.

One of the most obvious environmental differences between tropical and temperate regions is temperature, a variable that is strongly associated with development time in nematodes (Byerly *et al.* 1976; Anderson and Coleman 1982). It is notable in light of the geographic patterning of genetic variation outlined above that the tropically derived AF16 strain of *C. briggsae* has near-normal fertility at a temperature of 27.5°, whereas *C. elegans* is infertile at such high temperatures (Fodor *et al.* 1983; Grewal 1991). In addition, theoretical models suggest that selection pressures on hermaphrodite fecundity may differ in populations subject to rapid *vs.* slow development (Cutter 2004). Consequently, it will prove interesting to test for possible adaptations of geographically disparate *C. briggsae* isolates to temperature tolerance and other factors that correlate with latitudinal differences. Given the simple genetic basis underlying heat tolerance between some strains of *C. elegans* (Fatt and Dougherty 1963), this may prove to be a promising area of inquiry. The additional findings

that interspecific hybrid compatibility differs for temperate (HK104) and tropical (AF16) strains of *C. briggsae* mated to *C. remanei* strain EM464 (Baird 2002) and that these *C. briggsae* strains exhibit heritable differences in male ray pattern development (Baird *et al.* 2005) suggest that a variety of biological attributes may be amenable to dissection with genetic analysis.

The low effective population size estimated for *C. briggsae*, like that for *C. elegans*, implies that targets of weak selection will be acted upon inefficiently. For example, biased usage of alternative codons can be driven by natural selection, provided that effective population sizes are sufficiently large ($N_e = \sim 10^6$) (Akashi 1999). Codon bias is strongly correlated among orthologous genes of *C. elegans* and *C. briggsae* (Cutter and Ward 2005) and the codon bias of genes in *C. elegans* has been shown to be associated with gene expression, which has been interpreted as evidence of selection for translational efficiency (Stenico *et al.* 1994; Duret and Mouchiroud 1999; Duret 2000; Marais and Duret 2001; Castillo-Davis and Hartl 2002; Cutter *et al.* 2003b). How could selection result in these genomic patterns in the context of such low N_e? *C. briggsae*'s outcrossing ancestor likely experienced a much larger effective population size, so present patterns of codon bias might reflect historical patterns of selection. Even in the face of relaxed selection due to smaller N_e, codon usage bias is expected to decay very slowly, on the order of the mutation rate (Marais *et al.* 2004). Consequently, a relatively recent origin of selfing in *C. briggsae* (and in *C. elegans*) may account for the persistence of adaptive codon usage in these species. Indeed, the most divergent *C. briggsae* strains imply a common ancestor of perhaps 150,000 years ago (given $K_s = 0.0084$, $\mu = 9 \times 10^{-9}$, 60-day generation time), although such a rough calculation is subject to a number of caveats and is expected to underestimate the time since the origin of selfing (Cutter 2006). If this nominally greater coalescent time for extant variation in *C. briggsae* relative to similar calculations for *C. elegans* reflects an older origin of self-fertilization in *C. briggsae*, it might help explain the disparity in male longevity between these two species (McCulloch and Gems 2003) and the precedence of X-bearing male sperm in *C. briggsae* (Lamunyon and Ward 1997) as a consequence of a greater period of relaxed selection on *C. briggsae* males. A relatively recent origin of selfing in these species would have important implications for the interpretation of comparative sequence analysis between *C. elegans* and *C. briggsae*, because the vast majority of the millions of years of divergence separating them would have occurred in a state of outcrossing, rather than self-fertilization (Coghlan and Wolfe 2002; Stein *et al.* 2003; Kiontke *et al.* 2004). We also point out that rough calculations of the time to the most recent common ancestor between *C. elegans* and *C. briggsae*, on the basis of synonymous site divergence (Cutter and Payseur 2003a; Stein *et al.* 2003) and direct estimates of the neutral mutation rate in *C. elegans* (Denver *et al.* 2004; Keightley and Charlesworth 2005), suggest a more recent divergence than previous estimates that applied a molecular clock from non-nematode taxa (Coghlan and Wolfe 2002; Stein *et al.* 2003), due to the estimated worm mutation rate being much higher than for flies or mammals (Denver *et al.* 2004). Specifically, the data are consistent with a common ancestor of these species occurring 3.1–12.2 million of years ago (MYA) (range from 2.5% K_s percentiles, 1.2–20.7 MYA; Li 1997), depending on assumptions about generation time (30–90 days; faster generation time leads to more recent divergence).

The obligately outcrossing *C. remanei* is a closer relative of *C. briggsae* than either is to *C. elegans* (Kiontke *et al.* 2004), but it is not known where unidentified extant members of this genus might reside in the Caenorhabditis phylogeny. Given the large sequence divergence separating the currently described species in this genus, the identification of new sister taxa to available cultured strains will provide an important comparative context for dissecting the origin and evolution of breeding system transitions and other traits. For example, the recent isolation of a new gonochoric Caenorhabditis species (*C.* sp. 5, JU727; M. A. Félix, unpublished data), for which molecular data indicate a closer relationship with *C. briggsae* than with other members of this genus (Braendle and Félix 2006; Kiontke and Sudhaus 2006), will greatly facilitate comparative studies of development, breeding systems, speciation, and molecular evolution. As Caenorhabditis develops into a model for comparative genetics and genomics, it is imperative that current phylogenetic gaps are filled through the identification of new species.

The Caenorhabditis Genetics Center provided strains that were used in this study and Scott Baird made *C. briggsae* recombinant inbred lines available for use in mapping. We thank E. Dolgin and T. Johnson for helpful discussion and S. Baird and three anonymous reviewers for comments on the manuscript. This work was funded by the National Science Foundation with International Research Fellowship Program grant 0401897 to A.D.C. and by the Centre Nationale del la Recherche Scientifique and the Ministry of Research of France through a predoctoral fellowship to A.B. and a Biological Resource Center grant.

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Communicating editor: A. Villeneuve